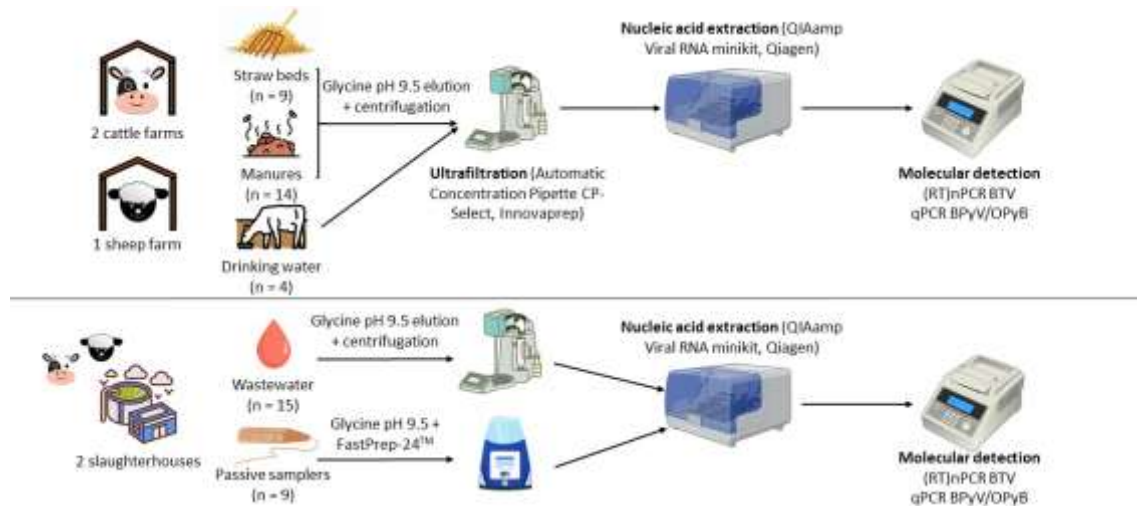


Supplementary materials: Environmental monitoring of viruses of veterinary concern by analyzing farm aggregated samples and slaughterhouse wastewater: a Bluetongue virus outbreak in Catalonia

Supplementary Figure 1



Supplementary Figure 1: Workflow of environmental sampling and viral detection. Aggregated samples from farms (drinking water, straw bedding, manure) and slaughterhouses (wastewater collected by passive and grab sampling) were processed by concentration (ultrafiltration or PEG precipitation), followed by nucleic acid extraction and molecular detection of BTV by nested (RT)PCR and of BPyV and OPyV by qPCR.

Supplementary table 1: Molecular assays used for viral detection, quantification, and typing, including primers, probes, and amplification conditions.

Bluetongue virus (BTV)							
Assay	Targeted segment	Primer/probe	Name	Sequence	Concentration (μM)	Amplicon (bp)	Reference
Broad nPCR	5	Forward	BTV_F1	GTTCTCTAGTTGGCAACCACC	0.25	259	Adapted from Katz et al. 1993
		Reverse	BTV_R1	GCAGCATTTTGAGAGAGCGA	0.25		
		Reverse nested	BTV_R2n	CCCGATCATACTTCTTCT	0.25		
		PCR program	1st PCR	95°C 3', 35 cycles 95°C 30", 58°C 45" and 72°C 1', 72°C 10'			
			2nd PCR	95°C 3', 35 cycles 95°C 30", 59°C 45" and 72°C 1', 72°C 10'			
Serotype 4 nPCR	2	Forward	BTV-4/2/1037-1055F	GCGCyTATCGCrATwGCyG	0.2	1071	Maan et al. 2012
		Reverse	BTV-4/2/2108-2090R	GAATTAGGTAAAYGATGCG	0.2		
		PCR program	1st PCR	95°C 5', 35 cycles 95°C 30", 55°C 30" and 72°C 45", 72°C 10'			
			2nd PCR	95°C 3', 35 cycles 95°C 30", 55°C 30" and 72°C 45", 72°C 10'			
Serotype 8 nPCR	2	Forward	BTV-8/2/669-691F	GAATTAGGTCGTAATCATCAAAT	0.2	562	Maan et al. 2012
		Reverse	BTV-8/2/1231-1207R	TCACTTCTCTATTCTATCGTAAG	0.2		
		PCR program	1st PCR	95°C 5', 35 cycles 95°C 30", 55°C 30" and 72°C 45", 72°C 10'			
			2nd PCR	95°C 3', 35 cycles 95°C 30", 55°C 30" and 72°C 45", 72°C 10'			

Bovine polyomavirus (BPyV)							
Assay	Segment	Primer/probe	Name	Sequence	Concentration (μM)	Amplicon (bp)	Reference
Broad qPCR	Non-segmented	Forward	QB-F1-1	CTAGATCCTACCCTCAAGGGAAT	0.4	77	Hundesä et al. 2010
		Reverse	QB-R1-1	TTACTTGGATCTGGACACCAAC	0.4		
		Probe	QB-P1-2	GACAAAGATGGTGTGTATCCTGTTGA	0.12		
		PCR program		95°C 10' and 45 cycles 95°C 15'' and 60°C 1'			
Ovine polyomavirus (OPyV)							
Assay	Segment	Primer/probe	Name	Sequence	Concentration (μM)	Amplicon (bp)	Reference
Broad qPCR	Non-segmented	Forward	Ov_qFI	TTCTCACAGCTGCAGACATTG	0.9	168	Rusiñol et al. 2013
		Reverse	Ov_qRI	TCCAATCTGGGCATAAGATTTG	0.9		
		Probe	Ov_qPI	ATGATTACCAAGCCAGACAGTGGG	0.225		
		PCR program		50°C 2', 95°C 10' and 40 cycles 95°C 15'' and 60°C 1'			

Supplementary table 2: Overview of samples collected during farm sampling campaigns and detection rates of bluetongue virus (BTV) and bovine/ovine polyomavirus (BPyV/OPyV) across farms and sample matrices.

		Manure	Straw beds	Drinking water	Swab	All samples
Cattle Farm 1	N° samples	7	3	1		11
	N° positive samples BPyV	5	3	1		9
	% + samples BPyV	71%	100%	100%		81.82%
	N° positive samples BTV	1	3	1		5
	% + samples BTV	15%	100%	100%		45.45%
Cattle Farm 2	N° samples	4	1	2		7
	N° positive samples BPyV	4	1	0		5
	% + samples BPyV	100%	100%	0%		71.43%
	N° positive samples BTV	2	1	2		5
Sheep Farm 1	% + samples BTV	50%	100%	100%		45.45
	N° samples	3	5	1	2	11
	N° positive samples OPyV	1	4	0	0	5
	% + samples OPyV	33%	80%	0%	0%	45.45%
	N° positive samples BTV	0	2	1	2	5
Total	% + samples BTV	0%	40%	100%	100%	45.45%
	N° samples	14	9	4	2	29
	N° positive samples B/OPyV	10	8	1	0	19
	% + samples B/OPyV	71.42%	89%	25%	0%	65.52%
	N° positive samples BTV	3	6	4	2	15
	% + samples BTV	21.42%	67%	100%	100%	51.72%

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